## Isolation of Tilletia caries from Infected Wheat Plants

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## ABSTRACT

Attempts were made to isolate *Tilletia caries* from leaves, ovarian tissue, and the upper three nodes and internodes of infected wheat plants. The pathogen grew only from infected ovaries and terminal internode tissue adjacent to the spike. It is hypothesized that most of the mature plant resists hyphal growth, and that the dikaryon develops only in localized areas rich in nutrients.

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Additional key words: bunt, fungi, teliospore, pathogen.

Most isolations of common and dwarf bunt (Tilletia caries [DC.] Tul. and T. controversa Kühn, respectively) were made from infected ovarian wheat (Triticum aestivum L.) tissue (5, 7). Few attempts have been made to isolate these pathogens from other parts of the wheat plant. The experiments described here were designed to determine those parts of the wheat plant that would consistently yield the common bunt pathogen, and whether isolates from culm tissue were as capable of producing teliospores in culture as were isolates from ovarian tissue.

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Germinating teliospores of *T. caries*, race T-1, were used to inoculate germinating wheat seeds of the susceptible cultivar, Red Bobs, and the resistant cultivar, Selection M68-112 (a Red Bobs backcross line carrying the *Bt*-9 gene for bunt resistance). The inoculated seeds were held in a moist chamber at 10 C for 1 week, transplanted, and moved to a 15-C growth chamber for 1 week, then grown to maturity in a greenhouse. Isolations were attempted from ovarian tissues, and from the upper three nodes and internodes as the heads began to emerge from the flag leaves. The inner tissue of these plant parts was carefully removed without the aid of surface disinfection, and placed on a chemically defined agar medium (1, 7). Also, leaves of Red Bobs, showing

chlorotic flecks indicating the presence of the pathogen (4), were surface disinfected and placed on the agar medium. Isolations were attempted from 30 Red Bobs plants and 20 Selection M68-112 plants.

In the susceptible cultivar, Red Bobs, the pathogen always grew from infected ovarian tissue and produced teliospores on the outside of the ovary and on the agar medium as it grew farther from the tissue. In about 70% of the isolation attempts, the pathogen also grew from terminal internode tissue located within 2 cm of the spike, and produced teliospores on that tissue and adjacent medium. Below that limit, in the terminal internode, the pathogen was rarely isolated. The pathogen was never isolated from the terminal node or sections of the culm below that point. The terminal node and its subjacent internode occasionally yielded an unidentified yeastlike organism. The pathogen was never isolated from the flecked leaves of Red Bobs. No evidence of common bunt, either hyphae or teliospores, was seen in or on any of the tissue samples from the resistant cultivar, Selection M68-112.

These results suggest that even in severely infected, susceptible Red Bobs plants, the pathogen can be isolated only from the ovarian tissue and the terminal internode tissue immediately below the spike. The pathogen is either not present or present at very low levels in the other plant parts. If the pathogen were present throughout the plant, but existing merely as a few hyphal strands, isolation would be difficult because the hyphae would have to grow through areas of wounded plant tissue. When wheat tissue is cut, browning reactions characteristic of oxidized phenols commonly occur within a few days. These reactions might adversely effect the growth of a few hyphae. Anatomical investigations (2, 3, 6, 8) of bunt-infected, adult wheat plants have also demonstrated that bunt hyphae are rarely found in the lower culm and leaf tissue. Infected adult plants showed hyphae in the terminal internode only, and never in the lower nodes or internodes (6).

It may be hypothesized that even in highly susceptible cultivars, like Red Bobs, the major portion of the plant is resistant to hyphal growth, and the pathogen (dikaryon) is able to develop only in very localized areas, through which and into which the nutrients needed for seed development are channeled

All wheat plants are resistant to the monokaryons of the bunt fungi. Therefore, if a wheat cultivar, susceptible to the dikaryon, can in some parts of the plant cause the dikaryotic hyphae to revert to its component monokaryons, then that plant part will be resistant. Factors responsible for causing dikaryons to revert back to monokaryons have not been investigated, but these could play a significant role in bunt resistance. Dikaryons are known to revert to monokaryons in sterile culture. It is noteworthy that monokaryons have never been isolated from infected wheat tissue.

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